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Mercury Accumulation Profiles and Their Modification by Interaction with Cadmium and Lead in the Soft Tissues of the Cichlid *Oreochromis aureus* during Chronic Exposure

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Mercury, cadmium and lead have no well known biological functions in the animal body, are normally present only in minute quantities, and are described as ultratrace elements (Lall 1989). Their toxicity is due in part to their competition with essential metals for binding sites and also their interference with sulfhydryl groups which are essential for the normal functioning of enzymes and structural proteins. Cadmium blocks sulfhydryl groups in enzymes and competes for sites with zinc and calcium. To a lesser extent, lead may replace calcium in structures and react with sulfhydryl groups, while mercury has a high affinity for sulfhydryl groups and is lipid soluble in its methylated form (da Silva and Williams 1991).

The chloroalkali industry is a major source of mercury pollution. When fish take up mercury, whether organic or inorganic, most of it accumulates in tissues in the organic form (WHO 1989). Minamata disease in humans was first reported in 1956 due to consumption of contaminated fish and shellfish from Minamata Bay (Mance 1987). The form of mercury responsible was found to be methylmercury, which being lipid soluble is much more toxic than inorganic mercury. In a case of Minamata disease in Kumamoto, some polluted fish and shellfish contained up to 20 $\mu g\ g^{-1}$ mercury based on wet weight (Kitamura et al. 1976). This emphasizes the importance of monitoring and assessing the mercury content of fish which are caught, or farmed, for human consumption. Since many commercial animal feeds contain a fishmeal component, there is a risk of contamination of farm animals intended for human consumption. Since Oreochromis aureus (Steindachner) is cultured in North and Latin America (Coche 1982), and other regions, it is a suitable model to use for studying the distribution of mercury in different tissues of food fish. Tissues which have a high content of mercury will be most

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dangerous from a toxicological point of view and could be removed in order to reduce the heavy metal content of fishmeal. Tilapias have been used effectively as constituents of pig food either directly or through fish silage or fishmeal (Balarin and Hatton 1981). Laboratory studies of heavy metal pollution often overlook the effects of exposure to more than one heavy metal at the same time. In episodes of environmental pollution, it is often the case that heavy metals occur in combination. In Jakarta Bay, high levels of cadmium were found together with mercury (Hutagalung 1989). For these reasons, the present study investigates the effects of exposure to combinations of mercury with cadmium or lead on tissue accumulation of mercury.

MATERIALS AND METHODS

Oreochromis aureus were bred in the Department of Zoology, National University of Singapore, and the fry were transferred to 120-L glass aquaria at a final stocking density of 300 fry per tank. The fry were observed, to ensure that they were disease-free, until they had attained an age of 6 wks. The use of antibiotics and other drugs was avoided as these can influence quantitative as well as qualitative characteristics of heavy metal distribution. At the end of the 6-wk observation period the fry were measured for total length, weighed and randomly assigned to plastic aquaria, each containing 25 L of tap water, at a density of 10 fish per tank. Ranges were 0.106-0.541 g for body weight and 20-33 mm for total length.

Each 25-L plastic aquarium was regarded as an experimental unit (n), defined as the unit of material to which an application of treatment is applied (Steel and Torrie 1960). Therefore only one of the surviving fish from each aquarium was selected for mercury analysis of tissues after the 45-day exposure period. Either 10 or 12 aquaria were used for each exposure concentration. This gave a maximum number of 10 or 12 fish analyzed per treatment. In some treatments an additional fish from each aquarium was selected for analysis of whole body mercury content. All chemicals were of analytical grade, purchased from Merck (Darmstadt, Germany). Stock solutions of 1 g L^{-1} cadmium, lead and mercury were prepared in double distilled water from cadmium chloride, lead (II) chloride and mercury (II) chloride. These were used to obtain the designated concentrations in each aquarium, with the exception of the controls. Nominal exposure concentrations for mercury alone were 0.05, 0.1 and 0.2 mg L^{-1}. The fry were also exposed to mixtures of 0.05 mg L^{-1} mercury with 0.05 or 0.5 mg L^{-1} lead, 0.05 mg L^{-1} mercury with 0.05 mg L^{-1} cadmium and 0.1 mg L^{-1} mercury with 0.1 mg L^{-1} cadmium. The fry were fed ad libitum on

a commercial pellet food and aquaria were examined several times a day for dead fish. Every 48 hr, 10 L of water were siphoned from each tank and this opportunity was taken to remove feces and uneaten food. Subsequently, 10 L of tap water containing the designated concentration of heavy metal were added to each tank. Control tanks received 10 L of water without heavy metals. The present system reflects the situation which might occur in environmental pollution incidents where the release of pollutants into the water is episodic, rather than continuous. After 45 days' exposure, the fish were sacrificed by decapitation. Tissues were dissected from the fish as described previously (Allen et al. 1988). Liver (hepatopancreas), brain, gill filaments, intestine and a block of muscle from the caudal peduncle were used. Tissues were digested using the wet oxidation method described by Gergely et al. (1977), except that concentrated nitric acid was used instead of concentrated sulphuric acid. Samples of fish liver, spiked with known quantities of mercury as mercury (II) chloride or methyl mercuric chloride, were run through the entire procedure to ascertain the percentage recovery of inorganic or organic mercury, respectively. The median recovery of methyl mercuric chloride, added to give a final concentration of 10 µg, was 89.9 % (n = 10). Mercury (II) chloride, added to give a final mercury concentration of 10 ug, gave a median recovery of 97.1 % (n = 10). The samples were analyzed for total (organic + inorganic) mercury content using a Perkin-Elmer (Model MAS 50B, USA) mercury analyzer system, based on the method of Hatch and Ott (1968) as employed by Gergely et al. (1977). Mercury content of acidified water samples, collected regularly from the experimental tanks, was measured. Lead content of the water was measured using a Hitachi Polarised Zeeman Graphite Atomic Absorption Spectrophotometer (Japan), while cadmium was determined by the technique of Inductively-Coupled Plasma Atomic Emission Spectrometry (ICP-AES) using a Plasmascan 710 with a Labtam 3000 computer (Labtam International Pte. Ltd., Melbourne, Australia). Calcium and magnesium in the tap water were determined by ICP-AES. The ranges were 9.10-11.83 mg L^{-1} for calcium and 0.83-1.00 mg L^{-1} for magnesium. Other water conditions were as follows; pH = 5.95-6.5, dissolved oxygen = 7.7-8.1 mg L^{-1} and temperature = 25-29^{o}C. Water hardness was in the range 26.14-33.65 mg L^{-1}.

Raw data were tested for normality with the G-statistic and homogeneity of variance using Bartlett's test, as described in Zar (1984). As the data did not meet the requirements of normality specified for the use of parametric statistical tests, nonparametric statistical procedures were used. For comparisons between one test group and one control group, the Mann-Whitney Test

(two-tailed; $\alpha = 0.05$) (Zar 1984) was employed to test equality of medians. Similarly, for comparisons involving 3 or more independent groups, the Kruskal-Wallis One-Way Analysis of Variance (Zar 1984) was used with the appropriate nonparametric multiple comparison tests (Zar 1984; Equations 12.27 and 12.28).

RESULTS AND DISCUSSION

Ranges of cadmium in the water were 0.093-0.102 and 0.045-0.052 mg L^{-1} for nominal concentrations of 0.1 and 0.05 mg L^{-1} cadmium. Ranges of lead in the water were 0.297-0.495 and 0.040-0.049 mg L^{-1} for nominal concentrations of 0.5 and 0.05 mg L^{-1} lead. Ranges of mercury in the water were 0.188-0.197, 0.067-0.095 and 0.036-0.046 mg L^{-1} for nominal concentrations of 0.2, 0.1 and 0.05 mg L^{-1} mercury. Lead and mercury were not detected in the control aquaria, while cadmium was 0.001 mg L^{-1} or lower.

Exposure to 0.05, 0.1 and 0.2 mg L^{-1} of mercury alone caused significant ($P < 0.05$) increases in the mercury content of all tissues analyzed. With 0.05 mg L^{-1} mercury (Table 1), liver, brain and gill filaments contained significantly higher concentrations of mercury than caudal muscle.

Table 1. Effects of 45 days exposure to Hg (0.05 mg L^{-1}, 0.1 mg L^{-1}) on tissue Hg levels in *O. aureus*.

Tissue	Control	Hg = 0.05	Control	Hg = 0.1
Liver	0.04(12)	43.97*(10)A	0.16(10)	7.23*(10)B
	0.00-0.28	4.45-85.92	0.00-0.27	4.73-15.23
Brain	0.10(12)	17.35*(10)A	0.39(10)	8.30*(10)B
	0.00-0.34	9.06-27.96	0.00-1.44	6.36-15.52
Gill	0.01(12)	30.04*(10)A	0.10(10)	19.58*(10)A
filaments	0.00-0.45	14.18-37.47	0.00-0.42	12.33-30.57
Intestine	0.03(12)	9.86*(10)AB	0.08(10)	9.91*(10)AB
	0.01-0.11	6.04-22.78	0.00-0.92	4.93-20.86
Caudal	0.01(12)	3.69*(10)B	N.D.	N.D.
muscle	0.00-0.06	1.73-7.60		

* denotes significant difference from the respective control group ($\alpha = 0.05$). N.D. = Not Determined.
Numerators denote the median, denominators the range.
Values in parentheses represent the sample size (n).
Medians with the same letters within the same column are not significantly different ($\alpha = 0.05$).
Units for medians are µg mercury g^{-1} tissue fresh weight

Exposure to 0.1 mg L^{-1} mercury (Table 1) resulted in the gill filaments accumulating a significantly higher mercury concentration than the brain and liver.

Interactions between mercury and cadmium or lead on tissue mercury accumulation are shown in Table 2. Treated fish were found to have significantly elevated concentrations of mercury in all of the tissues, with the exception of caudal muscle from fish treated with mercury and cadmium (0.05 + 0.05 mg L^{-1}) which had a low median mercury concentration (0.62 µg Hg g^{-1} fresh weight). Interaction between mercury and cadmium (0.05 + 0.05 mg L^{-1}) gave significantly lower concentrations of mercury in caudal muscle than in the gill filaments and the liver. Interaction between mercury and lead (0.05 mg L^{-1} + 0.5 mg L^{-1}) resulted in a significantly higher level of mercury in the gill filaments than in the intestine or caudal muscle. The interaction between mercury at 0.05 mg L^{-1} and lead at 0.05 mg L^{-1} (Table 2) resulted in significantly less mercury accumulation in caudal muscle than liver, brain and gill filaments.

Table 2. Effects of 45 days exposure to Hg with Cd (0.05 mg L^{-1} + 0.05mg L^{-1}) and Hg with Pb (0.05 mg L^{-1} + 0.5 mg L^{-1}, 0.05 mg L^{-1} + 0.05 mg L^{-1}) on tissue Hg levels in *O. aureus*.

	Control	Hg = 0.05 Cd = 0.05	Hg = 0.05 Pb = 0.5	Control	Hg = 0.05 Pb = 0.05
1	0.10(12)	13.07*(10)A	4.60*(12)AB	0.04(12)	13.21*(10)AB
	0.02-0.18	1.73-35.27	1.76-19.29	0.00-0.28	4.38-28.37
2	0.26(12)	8.35*(9)AB	4.51*(12)AB	0.10(12)	14.69*(10)AB
	0.00-0.39	1.19-22.03	1.44-24.87	0.00-0.34	8.67-28.01
3	0.06(12)	10.64*(10)A	14.81*(12)A	0.01(12)	30.94*(9)A
	0.00-0.15	3.23-27.59	5.90-26.47	0.00-0.45	13.41-43.20
4	0.05(12)	5.41*(11)AB	2.74*(12)B	0.03(12)	7.55*(10)BC
	0.00-0.17	1.20-19.80	0.53-8.95	0.01-0.11	4.72-11.64
5	0.05(8)	0.62(5)B	3.73*(8)B	0.01(12)	3.49*(10)C
	0.00-0.15	0.30-3.35	0.73-5.51	0.00-0.06	1.50-4.32

1 = liver, 2 = brain, 3 = gill filaments, 4 = intestine, 5 = caudal muscle. For legend see Table 1.

Exposure to 0.1 mg L^{-1} mercury with 0.1 mg L^{-1} cadmium (Table 3) resulted in significantly elevated mercury levels in the liver, brain, gill filaments and intestine. Due to the high mortality of the treated fish only 4 specimens were available for analysis. No significant difference in mercury concentration was found between liver, brain, intestine and gill filaments. Exposure to 0.2 mg L^{-1} mercury (Table 3) resulted in the liver accumulating a significantly higher concentration of mercury than the intestine.

Whole-body mercury concentrations for exposure to 0.05 mg L^{-1} mercury, 0.1 mg L^{-1} mercury and mixtures of mercury and cadmium (0.05 + 0.05 mg L^{-1}) and mercury and lead (0.05 + 0.05 mg L^{-1}) are given in Table 4. Exposure

to both concentrations of mercury and the mercury/lead mixture resulted in carcass mercury levels which were significantly higher than the control levels. There was no significant difference in the mercury concentrations of the 4 treatment groups.

Table 3. Effects of 45 days exposure to Hg (0.2 mg L^{-1}) and Hg with Cd (0.1 mg L^{-1} + 0.1 mg L^{-1}) on tissue Hg levels in *O. aureus*.

Tissue	Control	Hg = 0.2	Control	Hg = 0.1 Cd = 0.1
Liver	0.10(12)	37.34*(12)A	0.16(8)	50.66*(4)A
	0.00-0.18	10.42-948.25	0.00-0.27	14.07-119.0
Brain	0.26(12)	20.58*(12)AB	0.34(8)	10.45*(4)A
	0.00-0.74	11.65-100.24	0.00-0.75	6.37-20.31
Gill	0.06(12)	25.96*(12)AB	0.11(8)	22.61*(4)A
filaments	0.00-0.42	0.72-77.24	0.00-0.42	12.33-30.57
Intestine	0.04(12)	12.87*(12)B	0.08(8)	18.56*(4)A
	0.00-0.17	5.71-37.96	0.00-0.92	12.81-29.61
Caudal	0.04(4)	19.03*(4)AB	N.D.	N.D.
muscle	0.00-0.06	16.07-22.35		

For legend see Table 1.

Table 4. Effects of 45 days exposure to Hg and mixtures of Hg + Cd and Hg + Pb on mercury levels in whole fish.

Control	Hg = 0.05	Hg = 0.1	Hg = 0.05 Cd = 0.05	Hg = 0.05 Pb = 0.05
0.00(20)B	7.32(6)A	16.88(6)A	6.38(4)AB	10.62(6)A
0.00-0.02	5.56-9.96	12.84-24.23	3.44-11.90	3.49-15.37

For legend see Table 1.

Edible parts of whole fish, and fish products, intended for human consumption should not exceed 1 μg g^{-1} fresh weight of mercury according to the recommendations of the West German Federal Health Agency (Ewers 1991). Some countries allow a tolerance level of only 0.5 μg Hg g^{-1} (Gergely et al. 1977; Tracey 1993). In the present study whole-body mercury concentrations were much higher than these recommendations allow for whole fish. Control fish were largely free from heavy metal contamination, the only source being the fish food used in the present study. Fimreite and Reynolds (1973) quote whole-body mercury concentrations in fresh fish from the Wabigoon-English River System as follows: Burbot = 24.8 μg g^{-1}; pike = 27.8 μg g^{-1}; walleyes = 19.6 μg g^{-1}. These values are higher than those obtained in the present study after 45 days exposure to mercury (Table 4). In the present study, exposure to mercury deposited most mercury in the gills at 0.1 mg L^{-1} and in

the liver at 0.2 or 0.05 mg L^{-1} mercury. Interaction between 0.1 mg L^{-1} cadmium and 0.1 mg L^{-1} mercury produced a more equal distribution of mercury in the tissues analyzed. Interaction between 0.05 mg L^{-1} cadmium and 0.05 mg L^{-1} mercury did not change the accumulation profile of mercury in the tissues. However, mercury concentrations were higher in all organs measured after exposure to 0.05 mg L^{-1} mercury alone compared with exposure to 0.05 mg L^{-1} mercury in the presence of 0.05 mg L^{-1} cadmium. It would be tempting to assume that competition between cadmium and mercury for binding sites within organs was responsible for the apparent reduction of mercury accumulation in the fish exposed to 0.05 mg L^{-1} mercury in the presence of cadmium. However, the true mechanism may be much more complex because exposure to 0.1 mg L^{-1} mercury alone resulted in lower tissue accumulations of mercury than exposure to 0.05 mg L^{-1} mercury alone, presumably because of higher mortality in the 0.1 mg L^{-1} mercury-treated group in which fish with higher body burdens of mercury would have died. Furthermore, exposure to 0.1 mg L^{-1} mercury with 0.1 mg L^{-1} cadmium resuted in higher concentrations of mercury in the liver, brain, gill filaments and intestine in comparison with exposure to 0.1 mg L^{-1} cadmium alone. Lead, when present with mercury, exerted a similar reductive effect on the accumulation of mercury. Interaction of 0.05 mg L^{-1} lead with 0.05 mg L^{-1} mercury reduced the mercury content of the liver, brain and intestine when compared with exposure to 0.05 mg L^{-1} alone. When the lead concentration was increased to 0.5 mg L^{-1}, the mercury content of all the tissues, with the exception of caudal muscle, was reduced to an even greater extent. The nominal exposure concentrations used in the present study are similar in magnitude to those reported in environmental situations. Hutagalung (1989) reported mercury concentrations up to 0.035 mg L^{-1} and cadmium concentrations as high as 0.45 mg L^{-1} in Onrust Waters, Jakarta Bay. Total lead concentrations as high as 0.529 mg L^{-1} were reported from the Rother river in Canklow, U.K. (Mance et al. 1984).

Trends occurring in metal interactions require further investigation as data for cadmium, mercury and lead interactions and their accumulation have not been presented for long periods of exposure (Sorensen 1991). In the present study the absolute metal concentration of tissues is important because of its contribution to animal and human heavy metal burdens. While many metal mixtures may have an additive effect on toxicity in acutely toxic concentrations, there is no indication of this occurring at low concentrations over long periods (Mance 1987). Since muscle is the most commonly consumed portion of the fish and contributes most to the mass of the fish, it is the muscle

concentration of heavy metals which has received most attention. Fishes are purported to accumulate very little lead and cadmium in the muscle compared with mercury (Phillips and Russo 1978). Under all exposure conditions caudal muscle accumulated less mercury than other tissues except for exposure to 0.2 mg L^{-1} mercury, the highest mercury concentration used. However, exposure to 0.05 mg L^{-1} cadmium with 0.05 mg L^{-1} was shown to reduce caudal muscle mercury content below the 1 µg g^{-1} level. Under these conditions a slight reduction in whole body mercury content was also observed. The accumulation of mercury was particularly high in the liver and gill filaments under all exposure conditions used in the present study. Removal of these tissues during processing could reduce the mercury burden in fish and fish products.

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